

CLAIMS

What is claimed is:

1. An isolated and purified, biologically active KCC3 potassium-chloride cotransporter polypeptide comprising:
  - 5 (a) a polypeptide encoded by a nucleic acid sequence as set forth in any of SEQ ID NOs:3, 5, 7, 9 and 15;
  - (b) a polypeptide encoded by a nucleic acid sequence having 75% or greater sequence identity to nucleotides 1-434 of SEQ ID NOs:3, 5, 7, 9 and 15;
  - 10 (c) a polypeptide having an amino acid sequence as set forth in SEQ ID NOs:4, 6, 8, 10 and 16;
  - (d) a polypeptide which is a biological equivalent of the polypeptide set forth in SEQ ID NOs:4, 6, 8, 10 and 16;
  - (e) a polypeptide which is immunologically cross-reactive with an antibody which is immunoreactive with a polypeptide comprising  
15 part or all of the first 90 amino acids of any SEQ ID NOs:4, 6, 8, 10 and 16; or
  - (f) a polypeptide encoded by a nucleic acid molecule capable of hybridizing under stringent conditions to a nucleic acid molecule comprising the first 434 nucleotides of any of SEQ ID NOs:3, 5, 7,  
20 9 and 15, or complement thereof.
2. The polypeptide of claim 1, wherein the polypeptide comprises a mouse KCC3 potassium-chloride cotransporter polypeptide or a human KCC3 potassium-chloride cotransporter polypeptide.
- 25 3. The polypeptide of claim 1, modified to be in detectably labeled form.
4. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 1.
5. The antibody of claim 4, wherein the antibody is capable of  
30 modulating the biological activity of the polypeptide of claim 1.
6. A hybridoma cell line which produces the antibody of claim 5.

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7. An isolated and purified nucleic acid molecule encoding the biologically active KCC3 polypeptide of claim 1.

8. The nucleic acid molecule of claim 7, wherein the encoded polypeptide comprises a human or a mouse KCC3 polypeptide.

5 9. The nucleic acid molecule of claim 7, wherein the encoded polypeptide comprises an amino acid sequence as set forth in any of SEQ ID NOs:4, 6, 8, 10 and 16.

10 10. The nucleic acid molecule of claim 7, further defined as a DNA segment including part or all of the first 434 nucleotides of any of SEQ ID NOs:3, 5, 7, 9 and 15.

11. The nucleic acid molecule of claim 7, further defined as positioned under the control of a promoter.

15 12. The nucleic acid molecule of claim 11, wherein said nucleic acid molecule is a DNA segment, and the DNA segment and promoter are operationally linked in a recombinant vector.

13. A recombinant host cell comprising the nucleic acid molecule of claim 7.

14. An isolated and purified biologically-active KCC4 potassium-chloride cotransporter polypeptide.

20 15. The isolated and purified biologically-active KCC4 potassium-chloride cotransporter polypeptide of claim 14, wherein the polypeptide comprises:

- (a) a polypeptide encoded by a nucleic acid sequence as set forth in either of SEQ ID NOs:1 and 13;
- 25 (b) polypeptide encoded by a nucleic acid having at least about 75% or greater sequence identity to a DNA sequence as set forth in either of SEQ ID NOs:1 and 13;
- (c) a polypeptide encoded by a nucleic acid capable of hybridizing under stringent conditions to a nucleic acid comprising a sequence or the complement of a sequence
- 30 as set forth in either of SEQ ID NOs:1 and 13;

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- (d) a polypeptide which is a biologically functional equivalent of a peptide as set forth in either of SEQ ID NOs:2 and 14;
  - (e) a polypeptide which is immunologically cross-reactive with antibodies which are immunologically reactive with peptides encoded by the nucleic acid sequences as set forth in either of SEQ ID NOs:2 and 14;
  - (f) a polypeptide encoded by a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having the sequence of either of SEQ ID NOs:1 and 13, or fragments or variants or complementary sequences thereof, under high stringency conditions; or
  - (g) a polypeptide comprising a portion of a polypeptide of a), b), c), d), e), or f).
16. The polypeptide of claim 14, wherein the KCC4 polypeptide comprises a mouse KCC4 potassium-chloride cotransporter polypeptide or a human KCC4 potassium-chloride cotransporter polypeptide.
17. The polypeptide of claim 14, modified to be in detectably labeled form.
18. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 14.
19. The antibody of claim 18, wherein the antibody is capable of modulating the biological activity of the polypeptide of claim 14.
20. A hybridoma cell line which produces an antibody of claim 18.
21. An isolated and purified nucleic acid molecule encoding a biologically active KCC4 polypeptide of claim 14.
22. The nucleic acid molecule of claim 21, wherein the encoded polypeptide comprises a mouse KCC4 polypeptide or a human KCC4 polypeptide.
23. The nucleic acid molecule of claim 21, wherein the encoded polypeptide comprises an amino acid sequence as set forth in either of SEQ ID NOs:2 and 14.

24. The nucleic acid molecule of claim 21, further defined as comprising the nucleotide sequence of either of SEQ ID NOs:1 and 13.

25. The nucleic acid molecule of claim 21, further defined as a DNA segment.

5 26. The nucleic acid molecule of claim 25, further defined as positioned under the control of a promoter.

27. The nucleic acid molecule of claim 26, wherein said DNA segment and promoter are operationally inserted into a recombinant vector.

10 28. A recombinant host cell comprising the nucleic acid molecule of claim 21.

29. An isolated and purified biologically-active KCC2 potassium-chloride cotransporter polypeptide.

15 30. The isolated and purified biologically-active KCC2 potassium-chloride cotransporter polypeptide of claim 29, wherein the polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO:11;

20 (b) polypeptide encoded by a nucleic acid having at least about 75% or greater sequence identity to a DNA sequence as set forth in SEQ ID NO:11;

(c) a polypeptide encoded by a nucleic acid capable of hybridizing under stringent conditions to a nucleic acid comprising a sequence or the complement of a sequence as set forth in SEQ ID NO:11;

25 (d) a polypeptide which is a biologically functional equivalent of a peptide as set forth in SEQ ID NO:12;

(e) a polypeptide which is immunologically cross-reactive with antibodies which are immunologically reactive with peptides encoded by the nucleic acid sequences as set forth in SEQ ID NO:12;

30 (f) a polypeptide encoded by a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having the

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36. A hybridoma cell line which produces an antibody of claim 34.

38. The nucleic acid molecule of claim 37, wherein the encoded polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:12.

40. The nucleic acid molecule of claim 37, further defined as a DNA segment.

41. The nucleic acid molecule of claim 40, further defined as positioned under the control of a promoter.

42. The nucleic acid molecule of claim 41, wherein said DNA segment and promoter are operationally inserted into a recombinant vector.

43. A recombinant host cell comprising the nucleic acid molecule of claim 40.

44. A transgenic non-human animal having incorporated into its genome a nucleic acid molecule encoding a biologically active KCC2, KCC3 or

KCC4 polypeptide capable of modulating potassium-chloride cotransporter biological activity, the nucleic acid molecule being present in said genome in a copy number effective to confer expression in the animal of the KCC2, KCC3 or KCC4 polypeptide.

5           45.    An isolated and purified KCC2, KCC3 or KCC4 genomic DNA molecule.

          46.    The isolated and purified KCC2, KCC3 or KCC4 genomic DNA molecule of claim 45, further defined as comprising the nucleotide sequence of any of SEQ ID NOs:20-110.

10           47.    A method of producing an antibody immunoreactive with a KCC polypeptide, the method comprising steps of:

          (a)    transfecting a recombinant host cell with a nucleic acid molecule of any of claims 7, 14 or 37, which encodes a KCC polypeptide;

15           (b)    culturing the host cell under conditions sufficient for expression of the polypeptide;

          (c)    recovering the polypeptide; and

          (d)    preparing an antibody to the polypeptide.

          48.    The method of claim 47, wherein the polypeptide comprises a polypeptide as set forth in any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, and 113.

          49.    The method of claim 47, wherein the nucleic acid molecule comprises a nucleic acid molecule sequence as set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, and 112.

25           50.    A method of detecting a potassium-chloride cotransporter polypeptide, the method comprising immunoreacting the polypeptide with an antibody prepared according the method of claim 47 to form an antibody-polypeptide conjugate; and detecting the conjugate.

          51.    A method of detecting a nucleic acid molecule that encodes a potassium-chloride cotransporter polypeptide in a biological sample containing nucleic acid material, the method comprising the steps of:

- (a) hybridizing the nucleic acid molecule of any of claims 7, 14 or 37 under stringent hybridization conditions to the nucleic acid material of the biological sample, thereby forming a hybridization duplex; and
- (b) detecting the hybridization duplex.
52. An assay kit for detecting the presence of a potassium-chloride cotransporter polypeptide in a biological sample, the kit comprising a first antibody capable of immunoreacting with a polypeptide of any of claims 1, 14 and 29.
53. The assay kit of claim 52, further comprising a second container containing a second antibody that immunoreacts with the first antibody.
54. The assay kit of claim 52, wherein the first antibody and the second antibody comprise monoclonal antibodies.
55. The assay kit of claim 52, wherein the first antibody is affixed to a solid support.
56. The assay kit of claim 52, wherein the first and second antibodies each comprise an indicator.
57. The assay kit of claim 56, wherein the indicator is a radioactive label or an enzyme.
58. An assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a potassium-chloride cotransporter polypeptide, the kit comprising a polypeptide of any of claims 1, 14 and 29 that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.
59. An assay kit for detecting the presence, in biological samples, of a potassium-chloride cotransporter polypeptide, the kit comprising a first container that contains a nucleic acid molecule identical or complementary to a segment of at least ten contiguous nucleotide bases of the nucleic acid molecule of any of claims 7, 14 or 37.
60. A method to determine the presence or absence of a mutation conferring a pathological condition mediated by altered ion transport, said method comprising the step of analyzing a nucleic acid or protein sample for

the presence of a mutation in a human gene selected from the group consisting of KCC2, KCC3 and KCC4.

5        61.     The method of claim 60, further comprising the steps of amplifying nucleic acid molecules in said sample using a nucleic acid amplification method and primers that selectively amplify said KCC2 gene, KCC3 gene or KCC4 gene and identifying whether a mutation is present in said amplified nucleic acid molecule.

10       62.     The method of claim 60, further comprising the step of analyzing a protein sample for the presence of a mutation in a protein encoded by a human gene selected from the group consisting of KCC2, KCC3 and KCC4.

15       63.     The method of claim 60, wherein said human KCC2, KCC3 and KCC4 are further defined as comprising an amino acid sequence or a nucleotide sequence as set forth in any of SEQ ID NOs:1-110, 112-113, and 118-129.

20       64.     A method of screening candidate substances for an ability to modulate potassium-chloride cotransporter biological activity, the method comprising the steps of:

- 25            (a)     establishing test samples comprising a KCC2, KCC3 or KCC4 polypeptide;
- (b)     administering a candidate substance to the test samples; and
- (c)     measuring the interaction, effect, or combination thereof, of the candidate substance on the test sample to thereby determine the ability of the candidate substance to modulate potassium chloride cotransporter biological activity.

30       65.     The method of claim 64, wherein the candidate substance is further characterized as a candidate polypeptide, and further comprising the step of purifying and isolating a gene encoding the candidate polypeptide.

          66.     The method of claim 64, wherein the polypeptides are contained within cells in cell culture.

          67.     A recombinant cell line suitable for use in the method of claim 66.



68. A method for identifying a candidate compound as a modulator of potassium-chloride cotransporter biological activity, the method comprising the steps of:

- 5 (a) contacting a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of a KCC2-, KCC3- and/or KCC4- encoding gene and a reporter gene which is capable of producing a detectable signal;
- 10 (b) evaluating an amount of signal produced in relation to a control sample; and
- 15 (c) identifying a candidate compound as a modulator of potassium-chloride cotransporter biological activity based on the amount of signal produced in relation to a control sample.

69. The method of 68, wherein the reporter gene comprises KCC2, KCC3 or KCC4.

70. A method of modulating potassium-chloride cotransporter biological activity in a vertebrate subject, the method comprising the step of  
20 administering to the vertebrate subject an effective amount of a substance capable of modulating activity of a KCC2-, KCC3-, KCC4-polypeptide and combinations thereof in the vertebrate subject to thereby modulate potassium-chloride cotransporter biological activity in the vertebrate subject.

71 72. The method of claim 70, wherein the substance that modulates  
25 the KCC activity comprises an anti-potassium-chloride cotransporter antibody.

72 73. The method of claim 70, wherein the step of administering further comprises administering an effective amount of a substance that modulates expression of a KCC2-, KCC3-, or a KCC4-encoding nucleic acid molecule in the vertebrate.

30 74. The method of claim 73, wherein the substance that modulates expression of a KCC2-, KCC3-, or a KCC4-encoding nucleic acid molecule comprises an antisense oligonucleotide.

74 75. The method of claim 73, wherein the substance that modulates expression of the KCC2-, KCC3-, or a KCC4-encoding nucleic acid molecule comprises a ligand for a modulatable transcriptional regulatory sequence of a KCC2-, KCC3-, or a KCC4-encoding nucleic acid molecule.

5 75 76. The method of claim 70, wherein the vertebrate subject is a mammal.

76 77. A pharmaceutical composition comprising a therapeutically effective amount of a modulator of a biological activity of KCC2, KCC3, KCC4, and combinations thereof, and a pharmaceutically acceptable diluent or vehicle.

10 77 78. The pharmaceutical composition of claim 77, wherein the KCC2-, KCC3- and/or KCC4-biological-activity-modulator is selected from the group consisting of:

15 (a) a purified antibody which preferentially binds KCC2, KCC3, KCC4, combinations thereof, or a fragment or derivative thereof, and

(b) a polypeptide which interacts KCC2, KCC3, KCC4, combinations thereof, or a fragment or derivative thereof.

20 78 79. A method for modulating potassium-chloride cotransport in a vertebrate subject, the method comprising introducing to a tissue in said vertebrate subject associated with potassium-chloride cotransport a construct comprising a nucleic acid sequence encoding a KCC2, KCC3 or KCC4 gene product operatively linked to a promoter, wherein production of the KCC2, KCC3 or KCC4 gene product in the tissue results in modulation of potassium-chloride cotransport.

25 79 80. The method of claim 79, wherein the construct further comprises a vector selected from the group consisting of a plasmid vector or a viral vector.

80 81. The method of claim 79, wherein the construct further comprises a liposome complex.

30 81 82. The method of claim 79, wherein the KCC2, KCC3 or KCC4 gene product comprises a protein having an amino acid sequence as set forth in any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, and 113.

83. The method of claim 79, wherein the nucleic acid sequence is selected from the group consisting of:

- (a) a DNA acid sequence as set forth in any of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, and 112, or its complementary strands;
- 5 (b) a DNA sequence which hybridizes to a nucleic acid sequence as set forth in any of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, and 112 under wash stringency conditions represented by a wash solution having about 200 mM salt concentration and a wash temperature of at least about 45°C, and which encodes a KCC2, KCC3 or KCC4 polypeptide; and
- 10 (c) a DNA sequence differing from an isolated nucleic acid molecule of (a) or (b) above due to degeneracy of the genetic code, and which encodes a KCC2, KCC3 or KCC4 polypeptide encoded by the isolated nucleic acid molecule of (a) or (b) above.

84. A kit for detecting a polymorphism in a KCC gene in a subject, the kit comprising:

- (a) a reagent for detecting a polymorphism in a KCC gene in a biological sample from the subject; and
- 15 (b) a container for the reagent.

85. The kit of claim 84, further comprising a reagent for amplifying a nucleic acid molecule comprising a polymorphism in a KCC gene.

86. The kit of claim 85, wherein the amplifying reagent comprises a polymerase enzyme suitable for use in a polymerase chain reaction and a pair of oligonucleotides.

87. The kit of claim 84, further comprising a reagent for extracting a nucleic acid sample from a biological sample obtained from a subject.

88. A transgenic non-human animal having modified or deleted from its genome a nucleic acid molecule encoding a biologically active KCC2, KCC3 or KCC4 polypeptide capable of modulating potassium-chloride cotransporter biological activity.

89. An isolated and purified biologically-active *Xenopus* KCC potassium-chloride cotransporter polypeptide, wherein the polypeptide comprises:

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(a) a polypeptide encoded by a nucleic acid sequence as set forth in either of SEQ ID NO:112;

(b) polypeptide encoded by a nucleic acid having at least about 75% or greater sequence identity to a DNA sequence as set forth in SEQ ID NO:112;

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(c) a polypeptide encoded by a nucleic acid capable of hybridizing under stringent conditions to a nucleic acid comprising a sequence or the complement of a sequence as set forth in SEQ ID NO:112;

(d) a polypeptide which is a biologically functional equivalent of a peptide as set forth in SEQ ID NO:113;

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(e) a polypeptide which is immunologically cross-reactive with antibodies which are immunologically reactive with peptides encoded by the nucleic acid sequences as set forth in SEQ ID NO:112;

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(f) a polypeptide encoded by a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having the sequence of SEQ ID NO:112, or fragments or variants or complementary sequences thereof, under high stringency conditions; or

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(g) a polypeptide comprising a portion of a polypeptide of a), b), c), d), e), or f).

90. The polypeptide of claim 89, modified to be in detectably labeled form.

91. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 89.

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92. The antibody of claim 91, wherein the antibody is capable of modulating the biological activity of the polypeptide of claim 89.

93. A hybridoma cell line which produces an antibody of claim 91.

94. An isolated and purified nucleic acid molecule encoding a biologically active KCC4 polypeptide of claim 89.

95. The nucleic acid molecule of claim 94, wherein the encoded polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:113.

5 96. The nucleic acid molecule of claim 94, further defined as comprising the nucleotide sequence of SEQ ID NO:112.

97. The nucleic acid molecule of claim 94, further defined as a DNA segment.

10 98. The nucleic acid molecule of claim 97, further defined as positioned under the control of a promoter.

99. The nucleic acid molecule of claim 98, wherein said DNA segment and promoter are operationally inserted into a recombinant vector.

100. A recombinant host cell comprising the nucleic acid molecule of claim 94.